GAMETOGENESIS AND FERTILIZATION IN ALBUGO.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.

XXIX.

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(WITH PLATES I–IV)

[*Concluded from p. 169.*]

III. GENERAL CONSIDERATIONS.

OBSERVATIONAL evidence that kinoplasm and trophoplasm (Strasburger 1892) are true morphological elements of the cell has steadily increased, and striking experimental evidence has recently been adduced to confirm this conception (Hottes, *ined.*). In 1892 Strasburger proposed a theory of fertilization founded upon the assumption that sexual cells are incapable of development, owing to a kinoplasmic starvation. This theory was based partly upon observations on Ulothrix, and the relation of the cilia in sexual and asexual cells. Much confirmatory evidence has since been received, and in Strasburger's latest book (1900) the theory is developed more completely. As applied to sexually differentiated cells, the theory postulates kinoplasmic hunger in the female and trophoplasmic hunger in the male. The phenomena attending zonation in Albugo and Peronospora are capable of explanation in the light of Strasburger's theory, and in turn materially strengthen the theory itself. In Albugo and Peronospora the marshaling of the nuclei into a hollow sphere, a most conspicuous phenomenon, is quite inexplicable on the ground of atavism or phylogeny, nor can it have to do with wall-building. Why then do the nuclei habitually leave the ooplasm, apparently to perform no useful function in the periplasm, only to return and function as female pronuclei?

A study of the accompanying plates shows plainly that the periplasm is of a distinctly different character from the ooplasm.

The latter is typically very dense and alveolar, does not stain with gentian-violet but takes the orange G. The former stains darkly with the gentian-violet, and is filar, not alveolar. The processes leading to zonation may indeed be characterized provisionally as a differentiating of the oogonium into two regions, a periplasm rich in kinoplasm, and an oosphere rich in trophoplasm. This statement is borne out by all the positive characters of the trophoplasm and by the stain reaction and structure of the kinoplasm.

The nuclei are in mitosis and do not lose their membrane until zonation is sharp, nor do they reenter the ooplasm until their membrane is lost. The kinoplasmic nuclear membrane is apparently left in the periplasm, and its absence is evident during the second mitosis, thus resulting in that marked difference in character between the first and the second mitosis, which is illustrated in the figures, a difference which was noted in *A. Bliti* in my earlier paper (1899, p. 231). The nuclear membrane in the second mitosis is very thin or perhaps absent; the achromatic figure is weak and consequently often distorted and irregular. Every indication is that of an absence of kinoplasm.

Thus the behavior of both oogonial nuclei and cytoplasm confirms in a striking way the view that kinoplasm is important in sexual differentiation, and suggests that the nuclei pass to the periphery to rid themselves of superfluous kinoplasm, possibly to prevent parthenogenetic development in the oosphere. If this be the true reason for the migration of the nuclei, it logically follows that kinoplasm is not readily convertible into trophoplasm, at least not in the conditions that prevail in these oospheres.

In the antheridium a behavior complementary to that exhibited in the oogonium is seen. The antheridial protoplasm stains intensely with the gentian-violet before fertilization, but after fertilization the cytoplasm left in the antheridium fails to give this reaction. The antheridial nuclei as they lie in the tube possess a heavy membrane and stain darkly, giving every indication that they are rich in kinoplasm. In *A. candida* zonation is not

so marked, and the nuclei do not pass to the periplasm during division. In this species, however, there is a great preponderance of trophoplasm, owing to the highly developed coenocentrum, as well as to the fact that the trophoplasm of the whole oosphere is surrendered to one nucleus.

In general, the phenomena of oogenesis and spermatogenesis in Albugo afford remarkable confirmatory evidence for Strasburger's theory of fertilization. If the definite establishment of this theory should occur, botanists will come into more accord with those zoologists who accept the theory of Boveri that the sperm contributes the centrosome (kinoplasm), which is the one element needed by the egg to restore its capacity for division. Possibly the results of Klebs (1896), Loeb (1899), Nathansohn (1900), and others who have artificially induced parthenogenesis, may be explicable in the light of this theory, since it is at least conceivable that the environmental conditions which are supplied in these experiments may be identical with those which favor or retard the development of kinoplasm in the cell. Indeed, the results already attained by Hottes (1900) point in this direction.

No definite separating membrane can be detected in any of the species at the time of zonation, although the delimitation of periplasm and ooplasm is very sharp. Analysis of the condition shows that the differentiation is solely dependent upon the difference in character between the ooplasm and periplasm that has been described in previous paragraphs. It is outside of the ooplasm that the nuclei accumulate, and here in *A. Bliti*, *A. Portulacae*, and *A. Tragopogonis* they divide, some of the daughter nuclei returning to the oosphere.

The plasmoderma is formed at about the time that the primary oospheric nuclei renter, and at a period slightly later evidence of plasmolysis may be found. It appeared possible from some conditions seen in *A. Bliti* (Stevens 1899, *figs. 65–67*) that the nuclei might take some part in the formation of the new plasmoderma, but critical study shows that no constant relation is maintained between mitosis and plasmoderma formation. The

plasmoderma appears to arise directly from the cytoplasm in a manner closely resembling that which Mottier (1900) describes as a rearrangement of alveolar planes. The alveolae are here so small that it is impossible to assert with certainty that the processes are identical.

Simultaneous division of the nuclei in oogenesis and spermatogenesis is a phenomenon of wide distribution among the algae and fungi. The simultaneity itself is not remarkable, since it is frequently characteristic of multinucleate masses of cytoplasm, as endosperm nuclei of angiosperms, latex vessels (Pirotta and Buscalioni 1898), and plasmodia. The simultaneity in oogenesis is, however, of quite a different nature. Numerous vegetative nuclei, probably of very different ages, accumulate in the rudimentary sex organs and are there cut off from the parent cell. These nuclei pass simultaneously into mitosis, while the nuclei in the vegetative mycelium do not do so.[5]

This simultaneous mitosis, while it may be regarded with Hartog[6] (1891, p. 23) as a "phylogenetic reminiscence," is still often something more, and in the case of Albugo it is apparently a step necessary to the sexual differentiation of the gametes.

Whether a reduction in chromosomes occurs in connection with this gamete production is uncertain. To be sure Berlese (1898) claims to count the chromosomes during mitosis and fusion, and to establish definitely that reduction occurs in germination. The nuclear phenomena which he describes are so different from the conditions seen by Wager (1896), Davis (1900), and myself (1899), that the evidence must be accepted with reserve. The distinct difference in character between first and second mitosis in Albugo is, as I have said in another part of this paper, probably due to change in kinoplasmic content.

[5] Frequently nuclei in the immediate vicinity of the oogonium show a slight tendency to divide, and may even attain to the spirem stage (Stevens 1899, *fig. 45*).

[6] We can only regard the nuclear divisions in oogonium and antheridium as phylogenetic reminiscences of the formation of gametes by cell division; the periplasm is thus equivalent to a number of degenerate gametes which have taken on the function of epispore formation; the multitude of gametes are sacrificed to the few.

In three species of Albugo there are two mitoses, in another species probably two, in the gametangia; while in Peronospora (Wager 1900) there is only one. In Sphaeroplea (Klebahn 1899) the antheridial nuclei divide repeatedly, while the egg nucleus does not suffer visible change. In Vaucheria, even if there is a division in the oogonium, as seems possible (Oltmanns 1895, p. 392), it is probably not a differentiating division, since all of the nuclei but one wander back to the parent filament, and are presumably capable of vegetative function. From these examples it appears that the number of divisions in both oogenesis and spermatogenesis varies in different species, and differs in the two sexes of the same species; while in some forms there seems to be no mitosis directly concerned in the genesis of the female pronucleus. These conditions render improbable the existence of a reduction in the number of chromosomes during gametogenesis in these algae and fungi. Oogenesis and spermatogenesis begin almost simultaneously for a given pair or group of sex organs, yet all efforts to correlate their inception with any development external to the organs themselves were vain. A comparative study of four species shows no constant relation between the male and female organs in the sequence of their development, which seems to proceed independently in each organ. It is not probable, as might at first seem, that the inception of the antheridial tube is caused by the presence of the oosphere, since *fig. 50* presents a case where the antheridial tube grew and functioned normally, yet without a parallel development in the oogonium, or indeed the existence of an oosphere.

The factor which determines how many primary oospheric nuclei shall enter the ooplasm is uncertain. Clearly the position of the nucleus during its first mitosis determines whether or not one of the daughter nuclei shall enter the ooplasm. Yet this position seems to be governed by no law, the greatest irregularity existing, as is general in cases of simultaneous division in multinucleate masses of cytoplasm. This irregularity is equally prevalent at the time of zonation (*figs. 2, 28*). It is quite

possible, therefore, that mere accident determines how many of the nuclei are to be so oriented as to contribute daughter nuclei to the oosphere. It is needless to attribute to the cytoplasm any special selective power which causes definite nuclei or a definite number of them to return, although such selection is exhibited in some plants. For example, in the oogonium of Vaucheria (Oltmanns 1895) one nucleus maintains its position near the beak, amid violent activities of the surrounding cytoplasm, while the chloroplasts and numerous other nuclei are withdrawn. In the oogonium of Peronospora, according to Wager (1900), one nucleus of the many is selected to reenter the ooplasm and function as the female pronucleus.

The elimination of supernumerary nuclei by digestion in the surrounding cytoplasm finds analogy in vegetative cells in the sieve tubes of Pinus (Strumpf 1898); and still more striking in the sexual cells of Achlya (Trow 1899, p. 156), where there is an average of ten times as many nuclei as there are eggs to be produced. The phenomenon is also analogous to that described by Rückert (1892) and Oppel (1892), where several male nuclei enter the egg, the superfluous ones degenerating and functioning as yolk nuclei. In Actinosphaeria Hertwig (1898) also describes a reduction from multinucleate to uninucleate condition before fertilization, which is essentially similar to that seen in Albugo, the supernumerary nuclei being dissolved in the surrounding cytoplasm. In this species, as in Albugo, the nuclei are all alike, showing no such differentiation as is common among the infusoria. The phenomena seen in the Fucaceae (Oltmanns 1889), particularly in *Himanthalia lorea*, may be classed in this same category. Here the cytoplasm in early stages contains eight nuclei, the number being later reduced to one by casting off the seven superfluous ones.

In Vaucheria (Oltmanns 1895) the reduction from a multinucleate to a uninucleate condition is effected in quite a different manner, by protoplasmic streaming, the supernumerary nuclei being carried back into the parent hypha. Thus the reduction occurs before the oosphere is differentiated, and not in the

ooplasm proper. Klebahn (1899) describes in Sphaeroplea a case where the reduction from the multinucleate to the uninucleate condition does not occur until after fertilization, even if then, there being several nuclei in the oosphere only one of which is fertilized. The nuclei which receive no sperms are recognized in later stages by their smaller chromatin content, but their fate on germination was not followed. Golenkin (1900) agrees with Klebahn in finding a multinucleate egg, one nucleus of which functions; but Golenkin says that the nuclei then all fuse into one. Such a condition offers serious difficulties of interpretation in the light of the present theories regarding the cell. Since uninucleate oospheres were present among the multinucleate ones, it is possible that the condition observed by Golenkin was pathologic, a view which is strengthened by the fact that he was unable to germinate the spores after two years' trial.

In Saprolegniaceae Trow (1895, p. 630, and 1898, p. 166) notes a clumping or possibly pairing of the nuclei as they degenerate. I have also noted this phenomenon in the foregoing pages. Yet this is in no way to be confounded with the process of general fusion as described by Hartog (1891, p. 25) and Golenkin (1900), since these writers derive a functional nucleus from the ultimate result of successive fusions, while in the case observed by Trow, and in that seen by the writer, the nuclei thus appearing to fuse are really in the process of degeneration.

Inasmuch as it has been possible in all cases to follow the parallel development of the oospheres, it can hardly be doubted that in *A. Tragopogonis* and *A. candida*, as in Achlya, the Fucaceae, etc., the supernumerary nuclei represent potential pronuclei, and that each nucleus in the oosphere or *A. Tragopogonis* and *A. candida* is homologous with one of the nuclei in the oosphere of *A. Bliti* or *A. Portulacae*.

The coenocentrum has to some extent been discussed in connection with the description of *A. Tragopogonis* and *A. candida*. It yet remains to compare the structure in the different species. In *A. Portulacae* it is least developed, consisting simply

of a large zone of darkly staining cytoplasm which contains at its center the alveolar (trophoplasmic) region. This region seldom contains a globule such as characterizes the other three species. In *A. Bliti* the structure is much more prominent and endures for a longer period. It is of more complicated structure than in *A. Portulacae*, owing to the presence of a distinct central globule. In *A. Tragopogonis* the coenocentrum is still more highly developed. The central globule seems to be formed by the trophoplasm of the central region, or rather by the coalescence of the contents of its vacuoles. This globule in a later period becomes granular, the granules staining like nucleoli. *A. candida* possesses the most highly developed coenocentrum which, while closely resembling that of *A. Tragopogonis*, differs in that from its earliest formation till near the end of its functional activity it is thickly beset with coarse granules (*figs. 13, 17*) that in size and stain reaction agree with the nucleoli of this species.

In its function as well as structure this organ advances in complexity in the series here presented. In *A. Portulacae* there is no extensive accumulation of nutrient material in the vacuoles of the trophoplasm. In *A. Bliti* this accumulation is marked. In *A. Tragopogonis* the central globule shows strong chemotactic attraction for the nuclei and serves as nourishment for one or more of them. In *A. candida* this function of nutrition reaches greater perfection, as is shown by the attachment of the nuclei to the coenocentrum rather than their mere approximation to it. The coenocentrum develops earlier in the more highly differentiated species, and thus by exerting its attractive influence upon the nuclei before zonation strongly influences ontogeny. It has likewise probably been an important factor in changing the general character of oogenesis in phylogeny.

The presence of the receptive papilla in the four species of Albugo, as well as in Peronospora (Wager 1900, p. 270), attests to its importance either in the present or in ancestral species. Young stages in the development of this structure show that the plasmoderma adheres to the wall immediately under the

developing papilla, and exhibits a granular cytoplasm at this point. The granulation probably indicates the presence of a cellulose enzyme, formed here to soften the wall which alters chemically, as is evidenced by its response to stains and by its swollen condition (Stevens 1899, *figs. 47, 50*). The antheridial tube at this stage of development is probably unable to penetrate the cellulose wall of the oogonium, and that duty rests with the female cell.

Perhaps this curious structure can better be interpreted by a glance at possible ancestral forms. In algal aquatic forms, in which the gametangia open into the water, each sex organ opens independently, the female usually first. If the Peronosporeae have been derived from some such ancestors and these habits have been retained, in species where the walls of the antheridium and oogonium are in contact the origin of the receptive papilla is clear. Gametangia are usually of greatest turgor at the time of opening; therefore, in the present case the bulging, consequent upon the softening of the partition wall, is from the oogonium toward the antheridium. The formation of the receptive papilla in Albugo occurs immediately before the maturity of the oosphere, precisely as does the analogous phenomenon in Vaucheria.

The term "receptive papilla" is a misnomer, since this is not in any morphological sense a receptive structure, nor is it homologous with the receptive spot of the egg. In one case the differentiated area is part of an egg and functions as a place of reception for the sperm (as the eggs of Sphaeroplea, Saprolegnia, Oedogonium, etc.); in another case it is a zymogenic region of the protoplast adjacent to the point where an opening is to be made in an oogonial wall, either to furnish exit for the female gametes or for the entrance of the male elements, and is homologous with the opening spot of sporangia generally, as Cladophora, Bryopsis, Sphaeroplea, and Oedogonium. In the Saprolegniaceae the papilla does not furnish the place of entrance for the antheridium tube, although in Albugo it does (Zopf 1890, p. 293). The two regions occasion no danger of

confusion in the case of the multiovulate (vieleiig) oogonia, nor in cases where the egg is clearly differentiated in a surrounding periplasm; neither should they in forms like Vaucheria and Oedogonium.

The migration of antheridial nuclei into the tube seems to be independent of the developmental condition and metabolic activity of the oogonium, and is probably due to a negative rather than positive chemotropism, possibly of such a nature as that suggested by Hartog (1888) for the sporangia of the Saprolegniaceae. The objections raised by Humphrey (1892) to Hartog's view do not find application here, since he was considering cases where the sporangium failed to expel any of its spores. In Pyronema, where a condition similar to that of Albugo often prevails, Harper (1900, p. 362) explains the migration of some antheridial nuclei and the passivity of others by assuming that "the chemotactic or other stimulus which leads the male nuclei to migrate through the tube to the oogonium would in this case be assumed to have exhausted itself when a number equal to the number of egg nuclei had reached the oogonium." Such an hypothesis does not appear adequate for Albugo, for to assume that a stimulus which can arouse a given number of nuclei to migrate cannot bring the same activity to a greater number presents serious difficulties, and the supernumerary males are not found *en route*, as would be the case if they were stopped when the female nuclei have consorts and the hypothetical stimulating agent has been exhausted. The failure of some nuclei to leave the antheridium is more probably due to a lack of irritability on the part of some sperms than to a lack of the stimulating substance.

The phenomenon of the passage of nuclei from the body of the antheridium into its tube is no more comparable to the seeking of the female by a sperm than is the emptying of an algal sporangium, or an antheridium of the mosses or ferns, or the passage of the nuclei and cytoplasm from a pollen grain into the tube, all of which are clearly independent of chemotactic influence originating in organs of opposite sex, inasmuch as

they can occur in the entire absence of the female. It is after expulsion from the paternal gametangium that the chemotactic influence of the female unit is exerted upon the males. In the ferns, mosses, and most algae the chemotactic influence of the female extends over a comparatively wide region. In the spermatophytes the area is much restricted, since the pollen tube opens near the oosphere. In the Albuginaceae, where the male gametangium opens directly into the female gametangium, the region over which this influence may be exerted is still more limited. Indeed it may be questioned whether the force which brings the nuclei of the coenogametes (Davis 1900) together in pairs is at all the same as that which brings gametes together in the open. The pairing of nuclei in multinucleate masses of cytoplasm is comparable to the pairing of the male and female nuclei after the sperm has entered the cytoplasm of the egg, as is readily apparent from a consideration of the conditions presented in the larger eggs, as Fucus. Pfeffer demonstrated that the gametes in the open are drawn together by chemotactic attraction, and Wilson (1900) assumes that a similar attraction brings the nuclei together in the cytoplasm of the egg. Apparently two different forces operate, one to bring the sperm to the egg and induce penetration, the other to bring the pronuclei together in the ooplasm. Conklin (1899) has already distinguished these as distinct factors, and attempts to prove that in some cases at least cytoplasmic currents are responsible for the movement of the nuclei in the cytoplasm. Such an explanation does not seem adequate for the multinucleate oosphere of Albugo, as it would involve complexity inconceivably great. An explanation resting on chemotaxis is more tenable.

Conditions where an antheridial tube has reached the oosphere after that organ has been fertilized by another tube (*fig. 48*), as well as cases where two tubes open into one oosphere (*fig. 51*), show there is no correlation between the number of female nuclei to be fertilized and the number of nuclei which pass from the antheridial tube. This is also emphasized in *fig. 50*, where a tube is opening into an oogonium

having no oosphere, and into which an abundance of nuclei are poured. From these conditions, as well as direct observation, it is clear that the number of males and females is not exactly equal. In *A. Tragopogonis* unpaired male pronuclei are sometimes seen in the fertilized oosphere, but they are eventually digested by the ooplasm. Similarly, those male nuclei which do not pass into the antheridial tube, and those lying in an antheridium which forms no tube, fail to resume vegetative function and may be found *in situ* in stages of degeneration. The inability of such nuclei to function vegetatively attests their sexual differentiation and accounts for their subsequent rapid elimination.

One observation may help to disclose the force which directs the antheridial tube in its penetration of the oosphere. Two abnormal oospheres were seen quite devoid of nuclei. In one of these only the plasmoderma was developed (*fig. 52*), in the other periplasm and ooplasm were separated by a wall. In both cases the only antheridial tube present had gone astray in the periplasm. The absence of oospheric nuclei and the misdirection of the tube in the same oosphere may be only a peculiar coincidence.

Immediately after the opening of the antheridial tube a wall is seen surrounding the oospore. This develops with great rapidity, often attaining considerable thickness before the pronuclei have begun to fuse. Later thick walls, composed principally of cellulose for the nourishment of the germinating spore, are laid down from the inside, and on the outside heavy brown walls which are characteristically corrugated.[7]

In teratological forms, which abound in all species of Albugo, antheridial tubes where they lie in contact with the periplasm are frequently seen coated with the characteristic pectiniferous deposit. This occurrence was noted by DeBary (1863), and

[7] For brevity I shall hereafter use the term *pectiniferous* to designate this characteristic brown coating, inasmuch as pectin seems to be the constituent which is present here and absent from the other parts of the fungus. From the literature at hand the deposit appears to be really a mixture of cellulose, callose, cutin, and pectin, although its actual composition is yet open to question (Magnin 1895, Zalewski 1883).

mention of it is frequently found in works on the morphology of the group. Occasionally I have found isolated balls of this pectiniferous deposit lying in the periplasm. These observations in a measure confirm the generally accepted belief that the outer wall is laid down by the periplasm, a view originally proposed by DeBary (1863).

Sometimes an antheridial tube penetrates an oogonium that has not yet differentiated periplasm from ooplasm (*fig. 43*). In such case it often becomes coated with the characteristic pectiniferous wall of the species. More frequently it is aborted, reaching a length not greater than one-fifth the diameter of the oogonium. In such cases the adjacent oogonial walls and the remains of the aborted tube receive a pectiniferous coat (*figs. 44–46*). These phenomena tend to prove that undifferentiated cytoplasm of the oogonium has the ability to form the pectiniferous layer, and that in the absence of the plasmoderma of the oosphere it is apparently a matter of indifference what plasmoderma is to receive the deposit, although the pectin is laid down in contact with or by means of some plasmoderma or tonoplast in all cases.

Oogonia containing many small pectiniferous spheres (*fig. 46*) are quite frequently found, but the origin of the spheres cannot with certainty be determined. They are always accompanied by an aborted antheridial tube, and it may well be that these pectiniferous spheres represent deposits upon the lining membrane of vacuoles, thus emphasizing the similarity between the tonoplast and the plasmoderma in accord with the view of DeVries and Pfeffer. The fact that an aborted antheridial tube is present suggests that a stimulus may emanate from the antheridium which arouses the protoplasm to pectin production. This idea receives further support when it is recognized that the formation of the pectiniferous deposit begins and is most prominent near the antheridial tube (*fig. 44*). It thus often results in the tube becoming incrusted in a pectiniferous wall. *Fig. 45* represents an oogonium prematurely penetrated by two antheridial tubes from opposite ends. These tubes have aborted, but

each is accompanied by a pectiniferous formation from which the middle region of the oogonium is exempt.

Occasionally, by the formation of the oospore wall, a supernumerary antheridial tube is pushed aside in the periplasm. It then swells until it assumes a club-shaped appearance as it presses against the wall. In such cases the pectiniferous layer is formed over the whole mass, consisting of the mass of sperm nuclei and the remains of the antheridial tube (*fig. 48*). Conditions like these result, in the ripe spore, in a structure which looks much as though an antheridium lying beside an oospore had been encased (*fig. 49*). Such malformations may be distinguished from antheridia by the still persistent oogonial wall, which would of course not include the antheridium, but do include these masses.

If emanations from the antheridial tube stimulate the cytoplasm of the oogonium to form the pectiniferous layer, why does not the tube become coated as it penetrates the oosphere? This question probably receives its answer in the fact that the ooplasm has not the power to form pectin. It produces cellulose deposits, but not even in teratological cases (*fig. 47*) does it give evidence of any ability to form the pectiniferous layer. It is also notable that the ability to deposit the characteristic thick cellulose layer and to accumulate the typical oily globules is limited to the ooplasm. It seems, therefore, that in the activities leading to zonation there is a differentiation of the cytoplasm, that a capability vested in the oogonial cytoplasm is lost to the ooplasm, and its manifestation in later stages becomes limited to the periplasmic regions.[8] Whether this differentiation consists merely in a shifting of partially elaborated products of metabolism or in a segregation of living cytoplasm into two regions, one possessing a different constitution from the other, must be left an open question.

The stimulating effect from the antheridium may be manifested when the tube has penetrated only half way to the center

[8] The figures of Magnin (1895, *fig. 11*) might at first glance appear to contradic this, but Magnin's figures represent callose which is really present both in inner and outer walls.

of the oosphere without emptying, the stimulating agent presumably passing through the wall of the tube when this becomes very thin. The conditions justify the hypothesis that the protoplasm of the oogonium, or the periplasm as the case may be, contains the food constituents needed for the rather extensive production of the pectiniferous deposit, and that the substance contributed by the antheridial tube is more properly comparable to an enzyme than a food. It is a stimulant to activity rather than a material to act or to be acted upon. The protoplasm of the antheridium of Albugo, moreover, seems never to produce pectin, thus favoring the hypothesis that the necessary food materials are limited to the protoplasm of the oogonium.

The antheridial tube in normal conditions opens near the center of the oosphere, from whence the stimulating agent diffuses outward, awakening no response in the ooplasm because this is incapable of response. When this stimulating agent reaches the oospheric plasmoderma it meets the periplasm, which immediately lays down the rudiment of the outer wall. The fact that the tube opens in the center of the oosphere explains the absence of pectin formation in the periplasm, since the large area of the oospheric plasmoderma is ample to receive all the pectin that is to be laid down. Moreover, the thickening wall probably retards the passage of the stimulating agent into the periplasm, so that the peripheral portion of the periplasm is not incited to formation of the pectiniferous layer, and therefore the oogonial wall receives no pectiniferous coat. An answer to the question originally propounded by Cornu (1872) "si ce plasma extérieur a la propriété de se déposer en couche membraneuse, sans être élaboré au préabable, pourquoi ne se dépose-t-il pas aussi sur les parvis d l'oogone?" is thus suggested.

Normally it is the periplasm that responds to the stimulus, and the periplasm is bounded by its own plasmoderma and contains its own nuclei. It is an independent unit distinct from the oosphere, although it is destined to sacrifice itself for the protection of the oospore in a manner analogous to the tapetal

cells in many pteridophytes (Strasburger 1889). Normally, therefore, the production of pectin in Albugo is analogous to the so-called secondary effects of fertilization commonly seen in the higher plants, since it is an effect manifested by a cell other than those directly concerned in the act of fertilization.

SUMMARY OF SECTIONS II AND III.

The processes leading to zonation may be regarded as the differentiation of an ooplasm rich in trophoplasm. The nuclei pass outward, possibly to leave part of their kinoplasm outside of the ooplasm, in order to lessen the possibility of parthenogenetic development. The antheridial nuclei give evidence of heightened kinoplasmic content.

The cell plate is formed, without the participation of the nuclei, by a rearrangement of alevolar planes.

The simultaneous mitosis in gametogenesis is a phylogenetic reminiscence, and was of value in ancestral forms in increasing the number of gametes.

No constant time relation is maintained between the phases of oogenesis and spermatogenesis, but each after its inception seems to proceed independent of the other.

The orientation of the nuclear figure determines which, and consequently how many, primary oospheric nuclei shall enter the oosphere. This orientation seems to be merely accidental.

The supernumerary nuclei are phylogenetically gametes, and their dissolution finds analogy in the Saprolegniaceae and Fucaceae, in Actinosphaeria, and in cases of physiological polyspermy.

The receptive papilla is the result of a softening of the oogonial wall by the oogonial contents, accompanied by high turgor in the oogonium. It is probably a vestigial character recalling an algal ancestry. It is a structure of the oogonium, and therefore is not homologous with the receptive spot, which is a differentiated region of the oosphere.

The migration of the sperms from the antheridium is homologous with the emptying of a sporangium, rather than with

the seeking of the female by a male. The number of antheridial nuclei which migrate into the oosphere bears no constant relation to the number of waiting female nuclei. The failure of some nuclei to leave is probably due to a lack of irritability.

Superfluous nuclei of either sex which cannot resume vegetative function degenerate.

The periplasm has the ability to form the pectiniferous deposit, but the differentiated ooplasm cannot. Emanations from the antheridial tube seem to be needed to stimulate the ooplasm to this activity. The pectiniferous layer is deposited on or by a plasmoderma or tonoplast.

The four species, *A. Portulacae*, *A. Bliti*, *A. Tragopogonis*, and *A. candida* constitute a series in which the coenocentrum increases in complexity, the receptive papilla decreases, and the number of functional nuclei decreases. Of these *A. Portulacae* is probably the most primitive, and *A. candida* the most highly specialized form.

The coenocentrum was an important factor in evolution from the multinucleate to the uninucleate condition of oosphere.

The division of the fusion nucleus before passing to the winter condition is a consequence of the uninucleate condition, and constitutes the initial step in germination.

Delay in the division of the fusion nucleus in a uninucleate oospore is associated with retarded and slow fusion of the sexual nuclei, and is explicable as a consequence of slowness in completion of the last steps of fusion.

The relation between Albugo, Peronospora, and Saprolegnia is emphasized by their cytological character, and all are probably derived from a common ancestor having a multinucleate oosphere. The derivation of Peronospora and Saprolegnia from the Chytridineae is rendered improbable.

Pythium is more closely related to the Albuginaceae than to the Saprolegniaceae.

The peripheral gathering of the protoplasm in the oogonium of Saprolegnia may indicate closer relation to Peronospora than to Albugo.

If the Phycomycetes are related to Vaucheria it is from a period before the attainment of the uninucleate oosphere by Vaucheria.

The coenogamete is homologous with some or all of the gametes of a plurigametic gametangium, not with the individual gametes of such a structure.

There is a remarkable agreement between Albugo and Pyronema in many details.

The coenogamete is a result of pushing the synplast habit from the vegetative body into the reproductive organs.

The synplast of the Phycomycetes is a unit in both morphological and physiological sense, although it is philogenetically the equivalent of many units.

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EXPLANATION OF PLATES I–IV.

All figures are from material killed in chrom-acetic acid and stained with Flemming's triple stain. The figures were sketched with the aid of an Abbé camera, using the Leitz $\frac{1}{16}$ objective, aperture 1.30, and oculars 1 and 4.

PLATE I. Albugo Portulacae.

FIG. 1. Early stage in massing of protoplasm; nuclei in advanced prophase; receptive papilla prominent. × 857.

FIG. 2. Later stage in oogenesis; nuclei near metaphase; ooplasm and periplasm of very different structure, but not sharply delimited. × 857.

FIG. 3. Zonation; nuclei in metaphase; ooplasm and periplasm sharply differentiated; coenocentrum prominent, consisting of a loosely vacuolate center surrounded by a denser, slightly granular region which stains darker with the orange G. × 857.

FIG. 4. Slightly later than *fig. 3;* primary oospheric nuclei entering oosphere; ooplasm and periplasm sharply delimited; ooplasm typically alveolar, staining very lightly with orange G; periplasm staining densely with gentian violet, filar in structure; three reentering nuclei show each a very weak but distinct polar ray; coenocentrum has disappeared. × 857.

FIG. 5. Primary oospheric nuclei before second division; receptive papilla very prominent; later than *fig. 4*. × 857.

FIG. 6. Receptive papilla open; oospheric nuclei in second mitosis. × 857.

FIG. 7. Antheridial tube in oblique section showing many nuclei and no walls; oospheric nuclei in second mitosis. × 857.

FIG. 8. Antheridial tube in section slightly oblique; sperm nuclei numerous, elongated; stage slightly older than in *fig. 7*. × 1366.

FIG. 9. Transverse section of the antheridial tube, showing its multinucleate character. Oosphere of about the age shown in *fig.* 7. × 1366.

FIG. 10. Nuclei pairing after opening of antheridial tube, a distinct wall surrounding oospore; remains of antheridial tube visible in periplasm; degenerating nuclei in antheridium. × 857.

FIG. 11. Fusion complete; stage before accumulation of oils and before the outer walls are complete. × 857.

FIG. 12. Portion of mature wall. × 857.

PLATE II. Albugo Candida.

FIG. 13. Very young coenocentrum showing that the granules pass in from the surrounding ooplasm; oosphere slightly younger than stage next shown. × 1366.

FIG. 14. Early oogenesis; oosphere roughly outlined by a withdrawal of the protoplasm from the oogonium wall, leaving only a loose periplasm behind; nuclei approximately in metaphase; coenocentrum well developed. × 1366.

FIG. 15. After first division; several nuclei clustered around the coenocentrum; some already gone to the periplasm. × 1366.

FIG. 16. Later than *fig. 15;* nearly all of the nuclei have retreated to the periplasm; those remaining in the ooplasm in mitosis. × 1366.

FIG. 17. Later than *fig. 16;* mitosis complete. × 1366.

FIG. 18. Very slightly later than *fig. 13;* nucleus in metaphase, elongated owing to attraction by coenocentrum; coenocentrum densely and coarsely granular. × 1366.

FIG. 19. Two nuclei attached to coenocentrum; one in late anaphase, the other in telophase; coenocentrum more dense than in *fig. 18;* oosphere similar to that shown in *fig. 14.* × 1366.

FIG. 20. Similar to *fig. 19;* one nucleus in late anaphase attached to coenocentrum. × 1366.

FIGS. 21, 22. One nucleus much enlarged attached to coenocentrum by a pseudopodium-like extension; all other nuclei lie in the periplasm. × 1366.

FIG. 23. Before fusion; male and female nuclei and a supernumerary nucleus near the coenocentrum. × 1366.

FIG. 24. Metaphase of first division of fusion nucleus. × 1366.

FIG. 25. Anaphase of first division of fusion nucleus. × 1366.

FIG. 26. An antheridial tube bearing two nuclei; the female nucleus lying in the ooplasm near the coenocentrum. × 1366.

PLATE III. Albugo Tragopogonis.

FIG. 27. Early stage of oogenesis; protoplasm collected in one central mass; nuclei approaching metaphase; a slight indication of the coenocentrum apparent in the center. × 857.

FIG. 28. Condition slightly later than *fig. 27;* oogonium just before zonation; nuclei approximately at metaphase in both oogonium and antheridium; no sharp separation between ooplasm and periplasm. × 857.

FIG. 29. Slightly later than *fig. 28;* anaphase of first division; daughter nuclei entering ooplasm; ooplasm and periplasm sharply differentiated. × 1366.

FIG. 30. Immediately after the first division; primary oospheric nuclei moving toward coenocentrum, two of them strongly elongated; differentiation between ooplasm and periplasm sharp; plasmoderma probably present and many periplasmic nuclei pressing against it; coenocentrum prominent. × 857.

FIG. 31. Slightly later than *fig. 30;* ooplasm and periplasm separated by definite plasmoderma; oosphere multinucleate; coenocentrum contracted to a spherical homogeneous globule surrounded by dense protoplasm; primary oospheric nuclei attracted by coenocentrum. × 857.

FIG. 32. Metaphase of second mitosis; several nuclei attached to the coenocentrum; periplasmic nuclei not dividing; plasmoderma well defined; coenocentrum staining darkly and not homogeneous. × 857.

FIG. 33. Antheridial tube nearly ready to open, bearing several nuclei; oosphere in same condition as shown in *fig. 32, i. e.*, nuclei in second mitosis. × 1366.

FIG. 34. Similar to *fig. 32;* nuclei in second anaphase; ten dividing nuclei discernible in this one section. × 857.

FIG. 35. One female nucleus lying beside the coenocentrum—somewhat larger than daughter nuclei of second division; general appearance of the oosphere much like that in *fig. 36.* × 1366.

FIG. 36. After the opening of the antheridial tube; definite wall surrounding oosphere; remains of antheridial tube in oosphere; female nucleus in resting condition much larger than in *fig. 35* and lying beside the remains of the coenocentrum, which has lost its characteristic form; sperm nucleus somewhat elongated. × 857.

FIG. 37. Male and female nuclei lying in contact, both in resting condition, enveloped by the remains of coenocentrum; male larger than in earlier stages; compare *figs. 35, 36.* × 1366.

FIG. 38. Similar to *fig. 37;* also showing degenerate nuclei from same oosphere. × 1366.

FIG. 39. Fusion nucleus and remains of coenocentrum. × 1366.

FIG. 40. Anaphase of first division of fusion nucleus. × 1366.

FIG. 41. Winter condition of spore, showing thirteen nuclei in one section. × 857.

FIG. 42. Receptive papilla; oosphere like that shown in *fig. 32.*

PLATE IV. Teratological forms from various species (× 1366 or 857, and slightly reduced in reproduction).

FIG. 43. Antheridial tube penetrating oogonium in which no oosphere has been differentiated; tube branched and heavily coated with pectin; pectin also on oogonial wall in neighborhood of antheridium. *A. Tragopogonis.*

FIG. 44. Oogonium in which no oosphere was developed; antheridial tube small; deposit of pectin on oogonial wall in neighborhood of antheridium; also isolated pectin deposits, presumably in vacuoles; large deposit on the remains of the antheridial tube. *A. Portulacae.*

FIG. 45. An oogonium similar to that shown in *fig. 44;* two antheridia in contact with an oogonium; deposits of pectin lining the walls and projecting into the oogonium in the neighborhood of the antheridium; isolated balls of pectin lying free in the oogonium. *A. Portulacae.*

FIG. 46. An oogonium which developed no oosphere; masses of characteristic pectin distributed through the oogonium; deposits of pectin on the oogonial wall adjacent to the antheridium. *A. Portulacae.*

FIG. 47. Teratological formation of inner wall; redrawn from sketch, slightly diagrammatic. *A. Bliti.*

FIG. 48. Antheridial tube lying against plasmoderma of an oosphere fertilized by another antheridium; oosphere showing five nuclei in this section; antheridial nuclei in dense cytoplasm in the extreme tip of the tube; pectin formation encasing both oosphere and the end of the tube of the supernumerary antheridium. *A. Portulacae.*

FIG. 49. Similar to *fig. 48;* an older condition, showing whole structure surrounded by the remains of oogonial wall. *A. Portulacae.*

FIG. 50. Antheridial tube opening into an oogonium which has developed no oosphere; many male nuclei passing out, tube cut slightly oblique. *A. Portulacae.*

FIG. 51. Two antheridial tubes, each bearing many nuclei, opening simultaneously into one oosphere; one in transverse, one in longitudinal section. *A. Bliti.*

FIG. 52. Oosphere fully differentiated and devoid of nuclei; antheridial tube astray in periplasm; periplasm and antheridium normal. *A. Bliti.*

FIG. 53. An oogonium containing two coenocentra; oosphere bilobed, one coenocentrum lying at the center of each lobe; metaphase similar to *fig.* 26. *A. Tragopogonis.*